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# NATIONAL SYMPOSIUM ON YEAST FOR ALCOHOL AND FAT PRODUCTION

**23rd & 24th September 1987**

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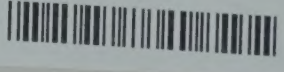

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# FOREWORD

Since centuries past, yeasts have been involved in the well being of man. Their role in the manufacture of fermented beverage, bread and industrial alcohol need not be over emphasised. These have become a part of man's daily life. In recent years, yeasts are being utilized industrially for the manufacture of various biochemicals, including proinsulin. And its use in the production of fat, of which there is an acute short supply in this country is being worked out all over the world.

Both alcohol and fat are considered to be very important in chemical and food industries. As these are in short supply, attempts to increase their production to meet the existing demand are going on, both at national and international levels, through various programmes.

Although ethanol production by yeast is at commercial level, it is still not anywhere near optimization for maximum economic yields. And fat production by yeast, though very promising, is yet to reach the stage of commercialization, mainly due to high comparative costs with vegetable oils at present.

Several programmes are centred around improving both ethanol fermentation and fat production by yeast. These include improving of strains of yeast for increased yields by various biochemical and genetic engineering techniques, utilizing alternate substrates for economic production, optimization of fermentation parameters for improved fermentation efficiencies safe disposal of waste waters and new economic means of product recovery.

The main objective of this symposium is to review the current Indian and world status on the research and development work on both ethanol and fat utilizing yeast and other microorganisms. Experts in the field have been invited to present the current status, discuss and recommend the future programme of action in the above areas. The proceedings of the symposium on "Yeast for Alcohol and Fat Production" will be published for dissemination of the knowledge amongst the participants and other important scientific industrial and academic organizations in the country.

The joint effort of the Indian Yeast Group, Association of Microbiologists of India (Mysore Unit) and the Central Food Technological Research Institute, Mysore, towards the success of this symposium is duly acknowledged.

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# PROGRAMME

23.9.1987

--- REGISTRATION ---

9.00 to 10.00 h.

--- INAUGURAL FUNCTION ---

10.00 to 11.15 h.

| | | |
|---|---|---|
| Welcome | : | S.P.Manjrekar |
| I.Y.G.Aims | : | P.Tauro |
| Theme of Symposium | : | S.C.Basappa |
| Inaugural Address | : | Yeast in Modern Biotechnology - P.R.Krishnaswamy |
| Release of Souvenir | : | B.N.Gopalakrishna |
| Keynote Address | : | Overview of ethanol and fat production by Yeast - N.K.Pandey |
| Chairman's remarks | : | S.K.Majumder |
| Vote of thanks | : | S.M.Aradhya |

.. HIGH TEA ..

11.15 to 11.30 h.

--- TECHNICAL SESSIONS ---

11.30 to 14.00 h.

SESSION I : Organisms and Substrates

| | | |
|---|---|---|
| Chairman | : | R.R.Davenport |
| Co-Chairman | : | P.Tauro |
| Rapporteurs | : | N.G.K.Karanth, and Vijaya Rao |

1. Newer organisms for ethanol and fat production ... N.K.Pandey
2. Yeasts in brewing and wine production ... P.Tauro
3. Simplified integrated yeast behaviour and identification methods ... R.R.Davenport
4. Utilization of substrates other than molasses for ethanol ... K.R.Sreekantiah
5. Zymomonas mobilis as an alternate organism for ethanol production ... S.C.Basappa

*** L U N C H ***

14.00 to 15.00 h

## SESSION II : Genetics & Biochemistry

15.00 to 17.00 h.

Chairman : M.R.Raghavendra Rao

Co-Chairman : R.Joseph

Rapporteurs : N.Chandrasekhara & A.A.M.Kunhi

1. Biochemical and genetic aspects of ethanol production and microbial fat ... R.Joseph
2. Maintenance of plasmids in the yeast Saccharomyces cerevisiae ... Pratima Sinha
3. Biochemical approaches to improvement in baker's yeast ... S.C.Basappa and G.Vijayalakshmi
4. Microbial tolerance to alcohols: role of cell membrane ... Rajendra Prasad
5. Genetic manipulation of non-conventional yeasts by conventional and non-conventional methods ... J.F.T.Spencer

I.Y.G. MEETING

17.00 to 18.00 h

E N T E R T A I N M E N T

18.00 to 19.00 h.

COCKTAILS AND DINNER

19.30 h.

24.9.87 SESSION III: Production Aspects

9.00 to 13.30 h.

Chairman : M.C.Bardia

Co-Chairman : N.G.Karanth

Rapporteurs : B.K.Lonsane and S.A.Jaleel

1. Current status of ethanol production in India ... M.C.Bardiya, S.Chandna and R.Hashia
2. Reactor designs for alcohol production ... N.G.Karanth
3. Treatment of waste water from industries ... V.Chandrasekhar
4. Engineering aspects of enhanced fat yields and productivities ... N.G.Karanth
5. Recovery of lipids and proteins from yeast biomass ... Z.Jacob

L U N C H

13.30 to 14.30 h

POSTER SESSIONS

(On All Aspects of Yeasts)

14.30 to 17.00 h.

PLEANARY SESSION AND RECOMMENDATIONS

17.00 to 18.00 h.

*** HIGH TEA --- 18.00 h ***

ANNALS OF A.M.I., MYSORE UNIT

The Association of Microbiologists of India (A.M.I.) is an official body of Indian Microbiologists, started in 1938. The Association is a forum for exchange of views and ideas among Indian Microbiologists to achieve better professional progress which, in turn, helps in the advancement of the country. Microbiological research plays a pivotal role in revolutionizing agriculture food industry and medicine. Considering the achievements made in these fields in the developed countries, Indian microbiologists have to intensify their efforts to keep pace with the fast developing field. Contributions by the A.M.I. (Mysore Unit) in this direction is not an insignificant one.

The Mysore Unit of A.M.I. was started in the year 1964 with the efforts of Drs. H.A.B.Parpia and K.R.Sreekantiah. In the beginning, it had members from the Microbiology Department of CFTRI and Post Graduate Department of Botany, Mysore University. By concerted efforts of the office bearers from time to time, the Unit started drawing members from the other Departments in CFTRI and from the other institutions in Mysore. Presently the Unit has about 100 members encompassing CFTRI, Mysore Medical College, Post-graduate Departments of Botany, Applied Botany and Zoology, Central Sericulture Research and Training Institute, Defence Food Research Laboratory, JSS College of Pharmacy and Kasturi Foods and Chemicals Limited. The Unit has ambitious plans to enrol all those who are involved in microbiological research and/or teaching in and around Mysore City as members. Untiring efforts by the office bearers and the constant encouragement by the fellow members would certainly culminate in achieving this goal.

Unit Activities

The Mysore Unit of A.M.I. is doing appreciable job in fulfilling the objectives of the Association. Seminars by microbiologists actively involved in research/teaching are arranged regularly. In this series, luminaries in Indian Microbiology such as late Drs. J.V.Bhat, and K.S.Gopalakrishnan, Dr.N.S.Subba Rao Dr.Sarbhoy, I.A.R.I., New Delhi; Dr.P.Tauro, H.A.U., Hissar.

Dr.T.N.Ramachandra Rao & Dr.V.Sreenivasa Murthy, Retd. scientists of CFTRI and Dr.D.V.Rege, U.D.C.T., Bombay have delivered lectures to share their rich experience with the members. Drs. W.J.Rennei, Agricultural Scientific Services; A.H.Rose,University of Bath; S.Bamburg, University of Leeds and F.I.Lamb, National Institute of Medical Research from U.K.; Dr.G.H.Davis, University of Queensland, Australia, D.T.Karki, Food Research Laboratory, Nepal, Dr.Anna Halasz, Hungary, Prof. H.G.Schlegel, West Germany, were some of the several exper: scientists from abroad, whose seminars were arranged in this Unit. Either once or twice in a year symposia are arranged on the topics of current interest In the Symposium held in 1980 on 'Microbial Ecology' at CFTRI Mysore, ten specialists spoke on Aerobiology, Marine Biology, Soil and Agricultural Microbiology and Food Microbiology. Half a day colloquim was organized in 1981, on 'Tissue Culture Systems' at CFTRI. A half a day symposium was arranged in 1985 on 'Recent Trends in Microbiology' at the Department of Applied Botany, University of Mysore. The topics encompassed silk worm diseases, research in infectious diseases, aerobiology, yeast production, plant protection and biotechnology. In 1986, a symposium on "Application of Biotechnology and Microbial Process" was conducted. The topics included genetic engineering, production of microbial proteins, whole cell immobilization fed batch fermentation and monoclonal antibodies. The members of A.M.I., Mysore Unit have thus been regularly appraised of latest developments in various aspects of Microbiology.

To help to update the knowledge of members on applied aspects of Microbiology, several field trips were arranged. In 1979, a visit to Kasturi Foods and Chemicals Ltd., Mysore was arranged. The members were made available of first hand information on baker's yeast production. A trip to Mysore Sugar Co. Ltd., Mandya, was arranged in 1980. Cane sugar production and production of bioethanol using molasses was looked at in detail. In 1981, a 'Microbiology Field Day' was organised. The members of the Unit were taken to Bangalore to visit Mysore Breweries, United Breweries, Indian Institute of Horticultural Research and Indian Veterinary Research Institute. These trips helped the members not only to

have a first hand knowledge about the Microbiology in application but also to enhance their enthusiasm and interest in the various aspects of Microbiology as well as the Unit activities.

To educate the common people and popularise microbiology, and to impart training to professionals in certain aspects of Microbiology, the Unit has been organizing Exhibitions and Workshops. The workshop in 'Food Hygiene and Sanitation' conducted in 1983 was a great success. Several food handlers and processors from various industries and catering establishments participated and the response was highly encouraging. Early this year an exhibition of the 'Role of Microbiology in Daily Life' was organized mainly to benefit common people. The importance of microbiology in improving the quality of human life, beneficial and harmful effects of microbes in daily life were depicted in the form of charts, diagrams, working models and specimens. The Exhibition evoked excellent response from the public especially from the student community. Presently, the workshop on 'Technology of Yeast for Distillers' followed by an Exhibition on 'Production and Utilization of Alcohol' has been arranged by the A.M.I., Mysore Unit in collaboration with CFTRI and IYG. Although it is arranged in a very short time, the response is very good. The Unit is forced to limit the number of trainees owing to the limitations in resource mobilization.

Although Microbiology as a branch of science is well developed and several institutions in and around Mysore City are utilizing microbial technology for various purposes, there was no course in Microbiology either at undergraduate level or at post-graduate level in the University of Mysore. The A.M.I. Mysore Unit took up the task of persuading the University to start a course at both the levels. The efforts were initiated as early as in 1979. The Unit assisted the University in chalking out a detailed syllabus for the course. As a result of these efforts, Microbiology specialization was introduced recently in the M.Sc. (Botany) course. The formal approval of the University has been obtained to commence Microbiology as one of the subjects in B.Sc. programme.

The A.M.I. Mysore Unit is proud of having several eminent microbiologists such as Dr.H.A.B.Parpia, Prof.K.M.Safeeulla, Dr.T.N.Ramachandra Rao, Dr.V.Sreenivasamurthy, Dr.S.K.Majumder, Dr.S.P.Manjrekar, Dr.K.R.Sreekantaiah, Dr.A.Ramalingam, Prof. H.Shekar Shetty and several others as its members. The Unit's contribution to the activities of A.M.I. at all India level is also well recognised. The Mysore Unit of A.M.I. along with CFTRI and DFRL successfully hosted 23rd A.M.I. Annual Conference at Mysore in November 1982. Two all India Presidents of A.M.I. were from this Unit. They are Dr.T.N.Ramachandra Rao (1975 & 1976) and Dr.V.Sreenivasa Murthy (1983). A great responsibility of bringing out the official scientific journal of A.M.I. was shouldered by Dr.S.P.Manjrekar, a member of the Mysore Unit, during 1984-86.

The present Office Bearers of the A.M.I. Mysore Unit are:

| | |
|---|---|
| President : | Dr.S.C.Basappa, CFTRI, Mysore |
| Vice President | Dr (Mrs.)Vijaya Rao, DFRL, Mysore |
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| Joint Secretary | Dr.H.S.Prakash, Dept. of Applied Botany, Manasagangotri, Mysore |
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With the constant guidance by the senior microbiologists of the Unit, endless efforts by the office bearers and whole hearted support and cooperation by the fellow members, the A.M.I. Mysore Unit is on its way to achieving greater strides in the field of microbiology and fermentation.

A Member of A.M.I.,
Mysore Unit.

# CONTENTS


FOREWORD

Annals of AMI, Mysore Unit. .... 1

SYMPOSIUM PAPERS

1. Overview of ethanol and fat production by Yeast - N.K.Pandey .... 7
2. Yeasts in brewing and wine production - P.Tauro .... 9
3. Utilization of substrates other than molasses for ethanol - K.R.Sreekantiah .... 15
4. Zymomonas mobilis as an alternate organism for ethanol production - S.C.Basappa .... 16
5. Biochemical and genetic aspects of ethanol production and microbial fat - R.Joseph .... 18
6. Biochemical approaches to improvement in bakers yeast - S.C.Basappa and G.Vijayalakshmi .... 20
7. Microbial tolerance to alcohols: Role of cell membrane - Rajendra Prasad .... 21
8. Genetic manipulation of non-conventional yeasts by conventional and non-conventional methods - J.F.T.Spencer .... 22
9. Current status of ethanol production in India - M.C.Bardiya,S.Chandna and R.Hashia .... 23
10. Reactor designs for alcohol production - N.G.Karanth .... 25
11. Treatment of waste water from industries - V.Chandrasekhar .... 26
12. Engineering aspects of enhanced fat yields and productivities - N.G.Karanth .... 27
13. Recovery of lipids and proteins from yeast biomass - Z.Jacob .... 27
14. Simplified integrated yeast behaviour and identification methods - R.R.Davenport .... 28
15. Maintenance of plasmids in the yeast Saccharomyces cerevisiae - Protima Sinha .... 29

POSTER SESSION I .... 31

POSTER SESSION II .... 39

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KEYNOTE ADDRESS

# OVERVIEW OF ETHANOL AND FAT PRODUCTION BY YEAST

N.K.Pandey
Hindustan Lever Research Centre
Andheri East, Bombay 400099

Yeast, a lower eukaryotic organism, has been found to be a suitable host for expression of prokaryotic and eukaryotic genes of diverse origin. The main properties for its suitability include the industrial experience available to handle it on a very large scale, the non-pathogenic nature, the ability to glycosylate the protein products and the well developed genetic exchange systems mediated by transformation, spheroplast fusion, rare mating, etc. Yeast is employed in a host of biotechnological processes in addition to brewing and baking. The present practice of brewing is fairly conventional, using yeast in batch or fed batch fermentations followed by distillation of alcohol. The substrate requirement, process parameters and flavour profile vary from product to product. The relevant improvements by application of genetics and bioengineering include continuous fermentation under semi-aseptic conditions in a tank or immobilised cell reactor with a stable, thermophilic isolate which is resistant to feedback inhibition by product[1]. The yeast cells can be immobilised by absorption, entrapment in hydrophilic gels and by covalent cross linking. Advantages of immobilised system over tank fermentation include higher reaction rate consequent to higher cell density, continuous operation without washout and release from end product inhibition as product alcohol is continuously removed[2,3]. In addition, costly equipment (fermenters, agitators, etc.) can be eliminated.

From the lists of fat producing bacteria[4], fungi[5], algae[6] and yeast[4] it appears that the latter are most promising for commercial production of fats and oils by fermentation. The major accumulating lipid of yeasts is triacylglycerol fraction (92% of total lipid of cell). Particularly SUS and SUU type triacylglycerols are predominant. The desirable properties of fat

and producer organisms as described by Professor C Ratledge are given as follows :

1. Fat should be of high commercial value and acceptable as a substitute for plant or animal fat.
2. Extracted cell residues should be saleable as protein supplement.
3. Organisms should produce significant amount of fat on biomass and should have a high sugar to fat conversion.
4. Organisms should grow rapidly on a variety of abundantly available substrates (carbohydrates as well as hydrocarbons).

For a strain to be useful for commercial production of fat, it should produce at least 50% fat on biomass (dry weight basis) and convert at least 20% molasses sugar to fat in a 2 to 3 day batch. A recombinant strain 63a, developed by us grew in pseudomycelial form in shake cultures. It accumulated greater than 50% w/w fat on biomass in glucose-urea-salts medium (C:N, 50:1) with sugar to fat conversion of 15%. In cane molasses-corn steep liquor-urea-salts medium (C:N, 20:1), the fat content was lower at Ca.30% w/w with 11-13% conversion. The productivity under optimal pH (6.5), temperature (30°C) and aeration (1 VVM during growth, 0.25 VVM during fat synthesis) with agitation (800 rpm) in fed batches on a 15 L Chemap fermenter was 4-5 g/l/d. Fermentation at pH 7.5 in potassium bicarbonate supplemented media influenced the fat production positively. The cells were hydrolysed with hydrochloric acid at 100°C and lipids extracted with hexane. The fatty acid composition of 63a lipids was similar to palm oil. The oil produced by *Lipomyces starkeyi* has also been found to resemble palm oil. The defatted meal had 26% protein containing mainly (mole %) glu, 4.1; asp, 3.8 ala, 2.8; pro, 2.3; leu, 2.1; lys, 1.9; gly, 1.8; ser, 1.5; thr, 1.2 and arg, 1.1. The yeast protein byproduct can be used as animal feed.

At 13% w/w yield on sugar and assuming 100% recovery, about 17 tonnes of molasses (45% sugar) would be required per tonne of oil costing Rs. 6,000 per tonne for molasses alone at present

prices (Rs.350/T including taxes). When the cost of fermentation, harvesting, extraction, recovery, refining and capital are added, the ex-factory price of oil will exceed Rs. 15,000 per tonne which is above the cost of edible oil in the country and the landed cost of palm oil. It is, therefore, clear that the opportunities for a microbial oil displacing conventional plant seed oils must be considered remote. It could come into its own when vegetable oil prices are high. With the strains developed so far, the economics is, unfavourable for all but the most expensive oils or for novel lipids whose synthesis has not been feasible previously.

In the long term, it should be possible to isolate and construct thermophilic, constitutive yeast strains capable of fermenting xylose, cellobiose, lignocellulosics and other abundant raw materials and with many desirable properties such as flocculence or floatation for the ease of harvesting cell and recycle.

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## YEASTS IN BREWING AND WINE PRODUCTION


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Wine and brewing industries constitute one of the largest potable alcoholic industries. Wine production in the world is presently estimated to be about 300 million hecto litres and the

production of beer is estimated to be about 700-800 million hecto litres annually. Further, these are beverages that are produced by fermentation but do not generally involve addition of undefined adjuncts nor distilled. Wines, by definition are the end products of alcoholic fermentation of fruit juices while beer is the end product of fermentation of a *grain* wort in combination with hops. Both are undistilled beverages and therefore, strict control over quality is absolutely essential. The main components in the production of these beverages are the substrate, the environmental conditions and the yeast culture used in the fermentation. The description herein will pertain mainly to the third component, namely the yeast.

The term wine yeast refers to several genera of yeasts which participate in the fermentation of grape juice. These include both the natural yeasts which are found in the surface of the grapes and are involved in the fermentation process or the yeasts that are added as pure cultures for controlled fermentation. Both types of yeast fermentations are practiced in the wine industry. In Europe, where nearly 80% of the wine is produced, wine fermentation is carried out mostly by the natural yeasts that are associated with the grapes, while in some other countries, grape juice is inoculated with defined strains of yeasts and allowed to ferment.

The most important wine yeasts belong to the genus *Saccharomyces* which are strongly glucophilic. Even during a natural fermentation, these yeasts predominate during active fermentation and during the later stages. There are several other yeasts which appear in sequence during the early stages but have little effect on the amount of ethanol produced but have a definite bearing on the quality of wine produced. There are yeasts which also causes spoilage and off flavours and these are also considered as wine yeasts. *Saccharomyces cerevisiae*, *Kloeckera* [illegible], *S. uvarum* and *S. pombe* are some of the common yeasts noticed. Species of *Saccharomyces* are strong fermenters. Cells are spherical, ovoid, ellipsoidal or elongated. They divide by multilateral budding and sporulation

can be induced since most are of higher ploidy. When sporulated, each ascus carries one to four ascospores. Under appropriate conditions, these can produce 10-18% ethanol. Other Species of Saccharomyces which can produce high amounts of ethanol include *S.chevalieri* (round to oval cells), *S.oviformis* (round to oval) *S.acidifaciens*(oval) and *S.bayanus* (oval to elongated and *S.baticus*. S.rosei also occurs in grape musts but is a low alcohol producer. S.rouxii and S.mellis are also encountered as spoilage organisms in sweet wines. Schizo saccharomyces pombe is generally not found but when found can produce both ethanol as well as deacidify wines rich in malic acid. Other yeasts found are Pichia which has short ellipsoidal to cylindrical cells that multiply by multilateral budding. On sporulation, 1-4 spored asci are formad. Some species form true hyphae. P.membranefaciens occurs in most grape musts. It does not produce more than 1-2% ethanol but can survive higher concentrations. It grows on the surface of young wines, as a continuous film.

Species of Hansanula such as H.anomala are important and these produce large quantities of ethyl acetate and other esters. They also produce upto 10% ethanol. They divide by multilateral budding with 1-4 spores per ascus.

Among Saccharomycedes, S.ludwigii is important. Cells are large in size (5-8 x 10-30u), cylindrical to sausage shape highly resistant to sulfur dioxide. These occur as spoilage organisms in highly sulfited wines although this can also produce 8-11% ethanol.

Species of Kloeckera such as K.apiculata dominate during early stages of fermentation upto 5-6% of alcohol although they can survive at higher concentrations of ethanol. They form volatile esters and volatile acids. Cell size is small and varies between 2.0-4.5 x 5-8u.

Among the fission yeast, Schizosaccharomyces pombe is important since this has the ability to convert malic acid into ethanol and carbon dioxide as well as produce 10-12% ethanol. This is useful in the deacidification of wines of high acidity but strains so far examined have not been found to produce a wine

of acceptable quality. The sexual stage is known in these fission yeasts and also contain 4-8 spores.

Among the nonsporulating yeasts, Candida and Torulopsis are important. C.mycoderma occurs in young musts and wines. Weakly fermentative (1-2% ethanol) but grow on wines at alcohol concentration higher than 6-8% forming a thin film over the surface. Torulopsis bacillaris, with oval to long cells is also found in wines, is poorly fermentative. Some produce volatile esters.

The appearance of natural yeasts during early stages of fermentation is not unexpected. However, in most wine producing countries species of Saccharomyces have established at all places of grape processing and as a consequence, even if the must is first treated with sulfur dioxide, subsequently the fermentation is brought about by the yeasts that are insensitive to this low level of sulfur dioxide.

In some countries such as US and Australia, wine yeasts of defined characteristics are used for inoculating the must. Before inoculation, the must is treated with sulfur dioxide and then inoculated with a pure culture of S.cerevisiae var ellipsoides. The level of inoculation is generally about 2%. The desirability of using a pure yeast culture in wine fermentation is debatable and controversial. There is however, general agreement that natural yeasts contribute flavour and aroma to the wine and it is also agreed that inoculation with wine yeasts will lead to sound and fully fermented wines.

When wine yeasts are used for grape juice fermentation the quality of wine produced by the different yeasts differs. For example, a Burgandy strain of S.cerevisiae var ellipsoides produces 3% more volatile acids than a Montrachet strain, There is also a difference in the amount of glycerol, volatile acids, fixed acids, aldehydes, $H_2S$ and higher alcohols produced by various strains of Saccharomyces cerevisiae.

The behaviour of yeasts during wine fermentation the rate and extent of fermentation etc. are factors controlled by temperature, type of fermentable sugar, pH, carbon dioxide pressure, the ethanol content, sulfur dioxide content etc. Nonetheless, the most

important single factor is the Yeast strain that will eventually determine the quality of wine produced.

The fermentation of ethanol from sugars is the principle end product of fermentation. However, in addition, several biproducts are also produced and these contribute to the taste and aroma of the wine. The maximum and minimum threshold limits for these components such as glycerol, higher alcohols, acetaldehyde, ketones, fixed acids and esters, hydrogen sulfide etc have been now established. Methanol found in wines is not from the fermentation but from the fruit which is produced by the demethylation of the pectin by the pectin methyl esterase. However, other aliphatic alcohols with more than 2 carbon atoms often termed as fusel oils and their quantity is determined by the yeast and the conditions of fermentation. For example, presence of excess valine in the must may lead to the production of excess isobutanol.

Rankine carried out an extensive investigation on various yeasts genera and found that each strain produced a characteristic distribution of fusel oil fraction. Further the formation of these fusel oils varies with the conditions since aerobic conditions and higher temperature allowed the production of more fusel oils. The production of aldehyde is more during early stages of fermentation and this is dependent on the yeast strain. When the aldehyde produced in excess earlier during the fermentation is not used by the yeast enzymes to produce ethanol, this could leak out of the cells. The production of aldehydes in sherries is a desirable characteristics and sherry yeast S.beaticus can produce high levels of acetaldehyde.

The production of glycerol in wines is also controlled by the yeast strain. At 25°C it has been found that the mean value of glycerol varies between 0.54 to 0.61 unit for several yeasts strains examined. An increase in the sugar content, or sulfur dioxide content etc leads to an increased level of glycerol in the wine.

During fermentation, a variety of volatile acids are also produced and these are products of wine yeasts. In the presence of air, some of alcohol is converted into acetic acid by yeasts.

The presence of hydrogen sulfide in wines is undesirable.

However some yeast strains depending on the composition of the must release considerable amount of this chemical into the wine. However, techniques are available to screen out yeasts that would produce less hydrogen sulfide into the wines.

Brewer'syeast :

The major difference between the wine fermentation and brewing is that brewing is never carried out by a natural yeast flora and therefore the yeast strain forms the major important component. in brewing. The quality of the beer, whether lager or ale, is determined partly by the wort composition and partly by the yeast. It is because of this that there is rigorous control in the maintenance of yeast quality for brewing industry.

The main characteristics looked for in brewing yeasts are the rate and extent of fermentation, growth, flocculation and flavour and aroma production. The beer yeasts belong to Saccharomyces carlsbergensis (uvarum) and S.cerevisiae. No other yeast is at present commercially used in brewing. The yeast can be a top or bottom fermenter depending whether a ale or a lager beer is produced. Since the brewing process includes a primary and secondary fermentation, the yeast mass after the first stage is cleaned and recycled. Flocculating yeasts allow a quicker separation of the yeast mass although in recent years the use of centrifuges has eliminated this requirement. There are a number of studies carried out to understand the process of yeast flocculation and it is believed that this phenomen is governed by a large number of yeast genes.

Brewer's yeasts have long been presumed to be genetically stable polyploids and that they posses superior fermentation characteristics. Inspite of their wide industrial usage, they suffer from some deficiencies which if corrected could greatly enhance their usefulness. For example, in brewer's yeast, the ability to metabolize starch and dextrins more efficiently would not only make the brewing process more efficient but also add a reduction in the cost factor in beer production. Addition of such properties from other yeasts such as S.diasticus, has been

attempted but not with much success. Also, beer fermentation is susceptible to easy contamination both by bacteria as well as by other yeasts. Contamination by other yeasts can be controlled by the introduction of the Killer factor from other yeast strains. The killer trait has been successfully transferred to a brewers yeast by transformation. Similarly, the trait to utilize the malto-dextrins have been successfully transferred to brewing yeasts. The success has however not been dramatic since the quality of beer produced by these genetically engineered yeasts has still to meet the desired standards.

Beer is also liable to spoilage by yeasts but this is not very serious problem since the natural inhibitors in the wort can control their growth.

## UTILISATION OF SUBSTRATES OTHER THAN MOLASSES FOR ETHANOL

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Ethanol or ethyl alcohol is in great demand for the manufacture of alcoholic drinks, and as a basic raw material for many chemicals. In India, ethanol of commerce is solely produced through fermentation of molasses. Eventhough the installed capacity is 1015 million litres per year, only less than half the quantity is produced annually. During the years of low sugarcane production, ehtanol production is also reduced and consequently there is a shortage. To overcome this shortage, it is necessary to utilise alternative substances for ethanol fermentation. Materials which can be utilised as fermentation substances are (1) starchy grains and tubers (2) forest products (3) saccharine plant material and (4) wastes from food processing industries. Relevant reports on this topic are reviewed and the processes developed discussed.

Important cereal grains that could be utilised for ethanol production are rice, wheat, sorghum, maize and barley. Utilisation of minor millets for alcohol production is more practical as these are not preferred by the majority of population as an item of food. Studies have also been carried out and processes developed to produce ethanol from tapioca, potato, and sweet potato. Residue from

starch factory, which form 20 to 30% of the tubers or grains processed can also be utilised for alcohol production. Sweet soybean has also been utilised for ethanol production in the USA. This crop is being introduced in this country only recently.

Among the forest products, mahua flowers is a promising substrate. Nearly 75 million litres of alcohol could be produced if all the material is collected and processed. Forest debris contain cellulose which can be converted into fermentable sugars and then to ethanol. During world war II, ethanol was being produced from wood chips by acid hydrolysis and fermentation. Even hemicelluloses can be fermented to ethanol. Microbes like *Pachysolen sp.*, *Candida sp.*, *Fusarium sp.* and a few bacteria have been utilised for this purpose. A soprophytic filamentous fungus *Monilia* sp. has been reported to convert cellulose to ethanol.

Fruit cannery wastes are also substrates suitable for ethanol production. Whey from dairy industry can be converted to ethanol by fermentation.

The technology involved in utilising non-conventional substrates for ethanol production will be discussed.

## ZYMOMONAS MOBILIS AS AN ALTERNATE ORGANISM FOR ETHANOL PRODUCTION

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The bacterium *Zymomonas mobilis*, which is used in the tropics to make pulque and alcoholic plam wines, appears to have considerable potential for industrial alcohol fermentation. Australian workers have shown that it possessed higher rates of sugar uptake and ethanol production than those of yeasts. It gave higher productivities (120-200 g/l/h), higher ethanol yields and lower biomass as compared to those of yeasts. *Zymomonas* metabolizes glucose via the Entner-Doudoroff pathway whereas yeasts convert glucose to ethanol via glycolysis. It grows anaerobically unlike yeast and does not require the controlled addition of oxygen to maintain Viability at high

cell concentrations. The ethanol tolerance of some strains is comparable to that of *S.cerevisiae*.

The genetic manipulation of *Zymomonas* should be simpler than yeasts. The genetic techniques could be applied directly for broadening the range of substrates utilised by this bacterium. It offers an attractive alternative to yeast for other genetic manipulations such as the selection of ethanol tolerant mutant.

At least three stress factors like thermal stress osmotic stress and alcohol stress are synergetic in their inhibition of fermentation in both *S. cerevisiae* and *Z.mobilis*. The investigations indicate that the cell membrane is the common site of action as evidenced by the high alcohol tolerance by the non-alcohol producing organism *Lactobacillus heterohiochi* which contained abundant membrane lipids. Genetic transformation of *Z.mobilis* by the alcohol tolerant genes of the *L.heterohichi* is underway in the USA. On the other hand, *Zymomonas* has a very limited substrate range and is capable of utilising only glucose, fructose and sucrose as carbohydrate sources. The other major disadvantage is the lack of industrial experience with this organism. However, several continuous-flow pilot plants are in operation using *Z.mobilis* for alcohol production in Japan, Canada, Australia, and New Zealand. And considering the improved thermal tolerance of *Z.mobilis*, this organism may prove especially useful in countries such as Brazil and India.

The advantages and disadvantages of *Z.mobilis* and *S.cerevisiae* have been worked out by German workers. Apart from the above advantages of *Z.mobilis*, over *S.cerevisiae*, the organism is at present beset with certain disadvantages such as more acid production 16 mm/1 and 12 mm/1 and for other byproducts (such as aldehydes, ketones, esters) 330 mg/1 and 280 mg/1 respectively. For industrial fermentation *Z.mobilis* seems to be inferior to *S.cerevisiae* because the advantage of higher ethanol and lower biomass production of the bacterium is disadvantaged by the decrease in pH from 6.3 to 3.3 during yeast fermentation thus making sterilisation of the medium unnecessary. The fermentation of acids and other by-products may be neglected in practice, since in contrast to previous assumptions they are easy to separate during the rectification.

Therefore, production of ethanol by *Z.mobilis* from molasses is inferior to that of *S.cerevisiae* because of low salt tolerance and low yields from sucrose. *Z.mobilis* needs improvement not only to low pH tolerance and high salt tolerance but also for lack of levan-sucrase enzyme to compete with yeast.

## BIOCHEMICAL AND GENETIC ASPECTS OF ETHANOL AND MICROBIAL FAT PRODUCTION

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The biochemical versatility of yeast came to light mainly during efforts made to understand the mechanism of ethanol biosynthesis in this organism. Ethanol production which was initially practiced as an art was subsequently subjected to detailed systematic studies revealing many interesting facets of yeast metabolism. Further, this has indicated a number of new possibilities to exploit yeast to make useful end products other than ethanol.

It is characteristic of the yeast to be able to thrive successfully under different physical and chemical environmental conditions, a fact which also explains how the organism can make different kinds of end products. It is well known that under microaerophilic conditions yeast can make either ethanol or glycerol as the major end product or a mixture of acetate, glycerol and ethanol. While ethanol is produced in a slightly acidic growth medium, in an alkaline medium the products could be the aforesaid mixture (acetate, ethanol and glycerol). Glycerol becomes the major product when acetaldehyde, the precursor of ethanol is chemically trapped. Like most other life forms, yeast utilises glucose primarily through the glycolytic pathway, but the few latter steps determine the type of end-product to be formed: and in this the noteworthy feature is the characteristic presence of pyruvate decarboxylase, glycerol-3P-NAD oxido-reductase and NAD dependant acetaldehyde dehydrogenase in yeast.

Besides fairly extensive present day knowledge of yeast metabolism practically nothing is known about the genetic relationship and regulation of metabolism. For instance, the various chemical signals (very likely) mediating switch in metabolism is yet unknown. Somewhat related to this question is the tolerance of yeast to elevated levels of ethanol on which our recent interest is focussed. Gross changes in the cytoplasmic membrane composition relating to alcohol tolerance are known, but the manner in which these changes are brought about (which conceivably involve repression/depression of certain genes) and the precise mechanism of alcohol tolerance are rather poorly understood. An interesting byproduct of these studies is the finding that in the presence of high levels of alcohol (or under other stress conditions) yeast makes different types of fatty acids of varying chain length and/or of unsaturation. Equipped with appropriate knowledge of the genetic and biochemical mechanism involved, it should be possible to custom make fatty acids of desired characteristics.

Most of the above discussion pertains to Saccharomyces cerevisiae, which is the chief industrial yeast employed in alcohol and baker's yeast manufacture. Certain other yeasts, some fungi and algae are endowed with the ability to overproduce fat (predominently triglyceride). Despite much available data on the biochemical mechanisms, involved, we are still far from understanding the actual reasons for fat over production. The enzyme ATP: citrate lyase which is detected only in oleagenous (fat overproducing) yeasts is perhaps a key factor. The phosphofructokinase and acetyl COA carboxylase whose levels are modulated could also favour oleogenecity. The first two enzymes contribute to high turnover of acetyl CoA, the fatty acid precursor at appropriate stage in culture growth cycle, while the third, acetyl COA carboxylase, favourably channels this precursor towards fatty acid biosynthesis. These steps are however, governed by the nutritional conditions of the culture. There is no data available on the nutritional conditions of the culture. There is no data available on the molecular mechanisms involved, particularly with respect to culture nutrition status vis a vis genetic regulation of fatty

acid biosynthesis. Adequate knowledge in this area would be necessary for successful exploitation of oleagenous yeasts for commercial production of fats.

## BIOCHEMICAL APPROACHES TO IMPROVEMENT IN BAKER'S YEAST

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Baker's yeast (Saccharomyces cerevisiae) is produced commercially all over the world by aerobic growth using cheaply available sugarcane or sugarbeet molasses. Although the production of wet compressed yeast (WCY) has almost always met with success, the production of Active Dry Yeast (ADY) has posed with certain problems of low activity and less stability during drying and storage. Hence, in order to improve the ADY in terms of yield, activity and shelf-life, several biochemical approaches have been made in this laboratory.

One of the important biochemical approaches was the content of trehalose a reserve disaccharide of yeast with regard to its (ADY) yield, activity and stability during storage. Synthesis, degradation and accumulation of trehalose under different physiological conditions of yeast growth were studied and found that accumulation takes place generally in stationary phase or ripening stage in fed batch fermentation perhaps due to gluconeogenesis. Since the yeast used in the experiment synthesised trehalose from glucose even during experimental phase of yeast, it indicated that the strain possessed 'MAL' gene and is a desirable quality of baker's yeast.

Addition of certain carbohydrates such as glucose,sucrose and maltose during ripening stage enhanced the trehalose content and has positive effect on the activity and stability of ADY. This was shown experimentally to be due to that these carbohydrates not only enhance trehalose content but also have a sparing action on trehalose. Mixing of certain other protective agents such as lecithin, glycerol enhanced the shelf-life of ADY probably by way of protecting the cell membrane.

Scanning electron microscope pictures did not show any difference between ADY cells treated with these protective agents and untreated cells. However, transmission electron micrographs showed clear line of membrane in treated cells as compared to undulated cell membrane in untreated cells indicating the membrane protection in treated cells.

Another reserve carbohydrate, the glycogen did not show any correlation either with yield or with activity and stability of ADY and its synthetic pattern was different from that of trehalose.

Even the structural carbohydrates of cell wall though varied in quantity within the cell cycle, did not show any correlation either with yield or with activity of ADY. However, it has been consistently observed that single cell (matured) stage is the correct stage of harvesting of cells that withstood well the drying conditions for a better ADY.

## MICROBIAL TOLERANCE TO ALCOHOLS: ROLE OF THE CELL MEMBRANE

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In addition to the importance of alcohol in pharmaceutical and brewing industries, the emergence of alcohol as a possible source of biofuel has further increased interest in its production. S.cerevisiae is the primary organism used for ethanol production. However, other microorganisms including Zymomonas mobilis, thermophilic bacteria and other yeasts are also being attempted as a cheaper way of ethanol production. Accumulation of alcohol in fermenting yeast represents a form of environmental stress, analogus to extremes in pH value and temperature. By understanding the effect of ethanol on S.cerevisiae and the biochemical changes that allow cell to adapt to alcohol, it is expected to gain insight into the regulation of cellular activities and relationship between the structure and function. The studies conducted so far have revealed that plasma membrane of yeast cells are the prime target of ethanol toxicity.

The fermentative activity of all ethanol producing organisms including S.cerevisiae declines progressively as ethanol accumulates in the surrounding media. The most ethanol resistant strains of yeast could only withstand a maximum of about 2 M ethanol concentration in the media. It is essential, therefore, that new strains which are more tolerant to ethanol are developed. The advancement in recombinant DNA technology has potential for combining the genetic traits from high resistant organisms with those from the best organisms for fermentative and substrate utilization. It is likely that multiple genes are invovled in membrane adaptation to alcohol tolerance and, therefore, a more detailed knowledge of mechanism of inhibition and changes in membrane lipid and protein composition which follow as an adaptation to enhanced ethanol concentration, is required.

The membranes and in particular plasma membrane are the prime target for ethanol inhibition in S.cerevisiae. The potency of alcohol as an inhibitor can be directly correlated with its lipid solubility, implying a hydrophobic site of action such as the cell membrane. The work from Rose's group have further shown that plasma membrane lipids are the targets for the interaction of ethanol. It was observed that the inhibitory effect of ethanol on viability of, and solute accumulation by S.cerevisiae was less marked when membranes were enriched with linoleyl as compared to oleyl residues. Accumulation of several nutrients is noncompetitively inhibited by ethanol. It is shown that the effect of ethanol on viability and solute accumulation could vary following fluctuation of plasma membrane lipids. The lecture will focus on the mechanism of ethanol toxicity in yeast and will discuss different approaches for the development of more ethanol tolerant strains.

## GENETIC MANIPULATION OF NON-CONVENTIONAL YEASTS BY CONVENTIONAL AND NON-CONVENTIONAL METHODS

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In recent years, yeasts other than those belonging to the species Saccharomyces cerevisiae and Schizosaccharomyces pombe

have become increasingly important in industrial process. Species such as Pichia stipitis, Hansenula polymorpha, Zygosaccharomyces rouxii, Saccharomyces exiguus, Torulaspora delbrueckii, Yarrowia lipolytica and others whose perfect stage is known, can be manipulated genetically by classical methods, but those belonging to the genera Candida (C.utilis, C.tropicalis, C.bombicola, C.zeylanoides, C. boidinil, etc.), Brettanomyces, Cryptococcus, Rhodotorula, and others of the different form genera, cannot be treated in this way. Some, such as Schwanniomyces and Debaryomyces spp.,which have a perfect stage, are still difficult to manipulate to conventional means. Genetic manipulation of these yeasts can be approached from two points of view; the first involving improvement of strains by cross-breeding within one species, and the second, the introduction of desirable genes from unrelated species and even from plants or animals.

## CURRENT STATUS OF ETHANOL PRODUCTION IN INDIA

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There are 150 distilleries in India producing Ethyl Alcohol utilizing cane molasses. The installed capacity of industry is about 1150 million litres per annum. The capacity utilization is nearly 50% every year because of the cyclic problems of raw material shortage and offtake of finished product. The utilization of Ethyl Alcohol is in the ratio of 60:40 by alcohol based industry in this country, is considered to be the traditional one. However, inspite of low value of final product and low margin of profit, the industry is moving ahead to modernize and update their technology for efficient utilization of molasses.

Raw material :

The major raw material for industrial alcohol production in cane molasses, the byproduct of sugar factories. In India, we are directly manufacturing plantation white sugar resorting to the vigorous boiling and chemical treatment for the better extraction of sugar thus leaving behind the molasses of poor quality

(low sugar, high unfermentables, calcium and yeast inhibitors). Furthermore, the storage of molasses in sugar factories in open pits and its dilution with rain water deteriorates its fermentability The sugar contents of molasses in India varies between 40 and 45% as compared to Brazilian molasses which is 50-55%. The distillers are left with no choice than to ferment molasses as made available irrespective of its composition and presence of harmful toxic compounds. Therefore, the comparison of recoveries of alcohol between Indian and Brazilian molasses has no validity irrespective of the type of process of fermentation employed.

Fermentation Processes :

Most of the Indian distilleries follow a conventional batch process, using either their own propagated yeast or baker's compressed yeast to obtain 6-8% (V/V) ethanol in 24-36 hrs. In the recent past, some of the distilleries have adopted latest technologies of fermentation, e.g., yeast recycling, semicontinuous process, biostill and continuous fermentation process. In these distilleries by the adoption of the latest technology the fermentation efficiency has gone up by 2-3 %. However, the practical problems and the results achieved by adopting some of the technologies in comparison to conventional batch process would be presented.

Distillation :

The steam is the main input in the distillation operation. Generally, the steam consumption in Indian distilleries varies from 2.5 to 3.0 Kg./litre of alcohol produced. In some distilleries, it is even more than 3.0 Kg. The theoretical steam consumption is about 1.8 to 1.9 kg./litre of alcohol, depending upon the alcohol concentration in wash. For reduction in the steam consumption, distilleries are putting up distillation columns of newly developed designs. Automation and instrumentation is another area where distilleries are fast entering for reducing the steam consumption and also for controlling the losses of alcohol during distillation.

Distilleries are putting strong emphasis on the adoption of waste heat recovery system. In condenser and heat exchangers, multipass design is being adopted for efficient removal of heat. Efficient type of heat exchanger are being utilized for heating feed wash and boiler feed water. In last one decade, there has been over-all decrease in the steam consumption in distillation mainly because of adoption of energy saving systems.

Effluent Treatment

Water management for distillery is of prime importance for reducing the amount of effluent. The use of cooling towers/ spray ponds have become a common feature for recirculation of process water. For the treatment of distillery effluent, a large number of process are being tried to evaluate their suitability under Indian conditions. For the recovery of methane gas from the distillery effluent, a number of distilleries are importing technical know-how from foreign countries. On the contrary, some of the distilleries are engaged in developing indigenous technology for the generation of methane gas from effluent. For the concentration and incineration technology of distillery effluent treatment, only one distillery has collaborated to put up a pilot plant to test out the process under Indian conditions because the total plant is highly capital intensive.

The comparative account of effluent treatment technologies would be presented.

## REACTOR DESIGNS FOR ALCOHOL PRODUCTION

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Fermentation of molasses to ethanol has been conventionally carried out in batch fermentors. Although continuous fermentation techniques have been known to give higher productivities, their application in industry has not been quite successful due to a number of factors. In the recent years however, several further developments have taken place, making some highly

efficient fermentation schemes economically feasible. Most techniques depend on the creation of a high concentration of cells within the fermentor and/or removal of the inhibitory product, ethanol. The methodology of creation of high cell densities or removal of ethanol gives rise to various reactor designs, which are discussed in this paper.

## TREATMENT OF WASTE WATERS FROM YEAST INDUSTRY

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M/s Kasturi Foods and Chemicals Ltd is producing compressed yeast to the extent of 150 tons per month using sugarcane molasses as the basic raw material. The production unit utilises about 400 $m^3$ water/day for clarification of molasses, yeast cream washing, equipment washing etc. These and other processes such as yeast separation, filter pressing etc, contribute to the waste water which is also about 400 $m^3$/day. The chief sources of waste water with high organic matters are : (1) from clarifier which contains sludge waste and has pH 4.2, B.O.D. 86,660 ppm, and total solids 5, 17, 400 ppm; (2) from yeast separator having pH 4.6, BOD 13,660 ppm, and total solids 35,260 ppm; (3) from filter press having pH 4.7, BOD 5000 ppm, total solids 6,2770 ppm and (4) from yeast cream wash having pH 5.2; BOD 4500 ppm, and total solids, 9,190 ppm. For the purpose of treatment, the wastes from all the sources are combined and combined effluent has pH 6.1, BOD 4,200 ppm, and total solids 7,490 ppm. The treatment schedule adopted is as follows:

a) The wastes from different sources are mixed in a distribution chamber and pH is regulated with time.

b) The waste water is then taken to holding tanks of 12h detention time.

c) Treatment in a series of primary anaerobic lagoons to bring down BOD to 775 ppm.

d) Treatment in secondary anaerobic lagoons to reduce the BOD to 450 ppm.

e) Treatment in extended aeration system with 12h detention time.

f) Storage in settling tanks to remove sludge. The settled sludge is removed to sludge pit and then to drying beds.

Steps e and f will reduce the BOD to 100 ppm.

g) The clarified effluent from settling tank is taken to polishing pond and then pumped to agricultural land for irrigation.

It is proposed to use sludge waste which is rich in organic matters for biogas production by anaerobic digestion, Cowdung will be used as the initial inoculum of anaerobic bacteria. The primary steps involved in the gas production are: (1) conversion of organic matters to organic acids by facultative bacteria and (2) conversion of organic acids to chiefly methane, carbon dioxide and ammonia. Methane can be separated and profitably used.

Production aspects of yeast and problems faced during waste treatment will be discussed in detail.

## ENGINEERING ASPECTS OF ENHANCED FAT YIELDS AND PRODUCTIVITIES

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The process of microbial lipid production often involves two phases, the biomass production phase and lipid accumulation phase. For a successful process economics, both the biomass productivity and lipid content are important. This paper critically looks at some of the important engineering aspects of enhanced fat yields and productivities.

## RECOVERY OF LIPIDS AND PROTEINS FROM YEAST BIOMASS

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Successful recovery of microbial lipid and proteins are dependent on a number of coordinated unit operations. Large

quantities of free fatty acids (10-20%) were reported to form during the process of microbial lipid extraction which is considered to be a serious problem when acceptability for its edible qualities are considered. However, a low quantity of free fatty acid content (2.3 to 3%) was observed with our laboratory procedures for R.gracilis. Regular and periodical modifications of old microbial protein recovery process have made the production of baker's yeast and its products more attractive and economic.

## SIMPLIFIED INTEGRATED YEAST BEHAVIOUR AND IDENTIFICATION METHODS

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ENGLAND.

Yeasts and yeast-like fungi can be difficult to study by microbiologists trained by conventional courses. Also methods for studying these organisms can be expensive, time consuming and often inadequate for proper assessment of problems. All too often many investigators with research projects either use a yeast without knowing its full properties or use a strain eg. Saccharomyces cerevisiae. This is the'E.coli' syndromes of producing a wealth of data on one organism and forgetting all others which may be completely different. In industry the pressure is greater and governed by commercial constraints, thus undue emphasis is placed on 'yeast and mould counts'and identification for everything: From quality control of project and sanitation to development of new commodities. Additionally the trend for most industries is to largely ignore most 'yeast and moulds' until there is a problem, then expert help is sought.

This paper sets out to show how simple inexpensive experimentation based on environmental concepts can provide behaviour patterns. These can be interpreted simultaneously for both technical assessment and recognition of yeast from a simple first step test a series of other simplified test can expand to give further technological data as well as identify the organisms. This environmental approach is an alternative system to current identification methods and has proved useful in many areas of research and development as well as quality control.

# MAINTENANCE OF PLASMÍDS IN THE YEAST SACCHAROMYCES CEREVISIAE : A REVIEW

Protima Sinha
Department of Biochemistry
Bose Institute, Calcuta-54

Extrachromosomal maintenance of plasmids, circular or linear, in baker's yeast, S. cerevisiae requires a replication origin that allows a plasmid to replicate autonomously. The replication origin could either be derived from the yeast endogenous plasmid, the 2 micron circle, or from yeast chromosomal DNA. DNA sequences isolated as promoting plasmid replication are called Autonomously Replicating Sequences(ARSs). Even though an ARS-carrying plasmid is able to replicate, it does not segregate equally to mother and daughter cells, being retained preferentially with the mother cells. The plasmid is therefore rapidly lost from cell cultures unless stabilised by DNA from yeast centromeres (CEN sequences). ARS/CEN plasmids are called minichromosomes in yeast and their stability, in part, depends upon nuclear genes controlling ARS or CEN functions.

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## POSTER SESSION I

### IYG - P(I)1 EFFICIENCY OF SOLID - LIQUID SEPARATION SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF TAPIOCA FLOUR TO ETHANOL

S.A.JALEEL , S.SRIKANTA, N.P.GHILDYAL, B.K. LONSANE AND N.G.KARANTH
Fermentation Technology and Bioengineering Discipline,
Cehtral Food Technological Research Institute, Mysore - 13

The process technology for production of ethanol from tapioca flour by simultaneous saccharification and fermentation involves the separation of wet cellulosic residue amounting to about 30% of the saccharified or fermented slurry. This can be done after the partial saccharification or after the fermentation using different solid-liqui separators. The results indicate a loss of 28-35% reducing sugars, (calculated from ethanol loss) when vibratory sieve, centrifuge and filter press were used in case of fermented slurry and 24-39% when partially saccharified hydrolysate was subjected to solid-liquid separation. The reducing sugar loss was lowest (24.5%) when solid-liquid separation was carried out in filter press from partially saccharified hydrolysate. The sugar loss can be further reduced by applying counte current extraction to the spent residue.

### IYG - P(I)2 KINETICS OF BATCH FERMENTATION OF CANE MOLASSES BY ETHANOL TOLERANT SACCHAROMYCES CEREVISIAE ISOLATES DESIGNATED AS Y-7 AND Y-10 AND STANDARD SACCHAROMYCES UVARUM STRAIN

B.SeetaRamaRao and H.SivaRaman
Division of Biochemical Sciences
National Chemical Laboratory Pune - 411008

The comparative kinetics of two ethanol tolerant yeast isolates *S.Cerevisiae* Y-10 and Y-7 are compared with those of brewer's yeast *S.uvarum* ATCC 26602 in the growth-associated fermentation of cane molasses in batch mode.

The kinetic parameters were calculated from the exponential phase of growth-associated fermentation. The results indicate that the isolates have more desirable characteristics than the standard *S. uvarum* strain for the fermentation of cane molasses to ethanol, specific growth rates ($\mu$), specific ethanol production rates ($q_p$) and sugar

uptake rates ($q_s$) being comparatively higher in the isolates than in *S. uvarum*. The values obtained for molasses containing 157 $gl^{-1}$ total sugar (glucose equivalent) were $q_p$=1.7; 1.6 and 1.1 $gg^{-1}h^{-1}$, $q_s$ = 3.3; 3.3; 2.14 $gg^{-1}h^{-1}$, final ethanol concentration ($gl^{-1}$)= 68; 65; 62, overall ethanol yield $Y_{p/S}$ ($gg^{-1}$) = 0.489, 0.474; 0.453 and theoritical ethanol yield = 95.9; 93.0; 88.7 respectively for S, Y-7, S Y-10, and *S. uvarum*.

## IYG - P(I)3 ENHANCEMENT OF FERMENTATION RATES OF CANE MOLASSES ON IMMOBILIZATION OF YEAST CELLS

A.V.Pundle, B.SeetaRamaRao, A.A.Prabhune, S.M.Kotwal and H.SivaRaman
Division of Biochemical Sciences
National Chemical Laboratory Pune-411008

Yeast cells immobilized by entrapment in a porous gelatin matrix have been whown to give higher specific ethanol productivities and better tolerance towards high salt concentration in molasses than the free cell suspensions under batch fermentation conditions. The experiments were carried out in batch mode using *Saccharomyces uvarum* ATCC 26602 immobilized in open-pore 20% gelatin matrix described earlier by us. H.SivaRaman, B.SeetaRamaRao, A.V.Pundle and C.SivaRaman. (Biotechnol. Lett. 1982, 4, 359 - 364). The specific ethanol productivities in the initial phase of fermentation of molasses containing 100 to 200 $gl^{-1}$ total sugars were calculated. This was done before product inhibition was evident. The values obtained with free and immobilized cells at 100 $gl^{-1}$ molasses fermentable sugar concentration were 0.6 $gg^{-1}h^{-1}$ and 0.74 $gg^{-1}h^{-1}$ for the free and the immobilized cells respectively; the corresponding values at the higher substrate concentrations of 150 $gl^{-1}$ and 200 $gl^{-1}$ being 0.5 $gg^{-1}h^{-1}$ and 0.8 $gg^{-1}h^{-1}$ and 0.36 $gg^{-1}h^{-1}$ and 0.7 $gg^{-1}h^{-1}$ respectively.

In all cases the immobilized cells gave higher specific ethanol productivities than free cell suspensions under similar conditions.

# IYG - P(I)4 CONTINUOUS ETHANOL PRODUCTION USING AGAR-IMMOBILIZED YEAST SYSTEM

K.R.Pillai, B.K.Gogoi and H.D. Singh
Biochemistry Division
Regional Research Laboratory, Jorhat,Assam

Agar was used as a medium for the immobilization of yeast cells. Unlike calcium alginate, agar immobilized yeast system was stable in presence of phosphate salts in the medium. A cell loading of 40-50 mg (dry cell equivalent) yeast cells per gram of wet agar beads was found to be the optimum. A 3-5% agar concentration and a 2.7-5mm agar bead diameter was found to be satisfactory for continuous ethanol production.

Using the agar immobilized whole yeast cell, fermentation parameters involved in ethanol fermentation have been studied and the performance of an immobilized packed bed column reactor system was evaluated. At an influent D-glucose concentration of $23gl^{-1}$, the maximum ethanol concentration obtained in the effluent was $96gl^{-1}$, at a mean residence time of 3.75h. The corresponding ethanol productivity and yield were $25.6gl^{-1}h^{-1}$ and 79% respectively. The average ethanol yield at lower D-glucose concentration (100-200g $l^{-1}$) was 90% of the theoretical. The maximum productivity obtained was $83gl^{-1}h^{-1}$ at $166.6gl^{-1}$ D-glucose medium at a mean residence time of 0.75h. The corresponding ethanol concentration was $62.5gl^{-1}$ with 73% theoritical yield. At a mean residence time of 1.25h the ethanol concentration in the effluent was $74gl^{-1}$ with 87% theor tical yield, but $59gl^{-1}hr^{-1}$ productivity. Using molasses as the substrate ($187gl^{-1}$ reducing sugar) the operational stability over a period of 65 days was studied. The performance of the reactor was found to be uniform during this period. The ethanol concentration in the effluent was $73.8gl^{-1}$ at a mean residence time of 1.6h and the productivity was $46gl^{-1}hr^{-1}$.

## IYG - P(I)5 EFFECT OF SKIM MILK ON ETHANOL PRODUCTION USING YEAST CELL RECYCLING IN CANE MOLASSES FERMENTATION

S.G.Patil, and B.G. Patil
Division of Biochemical Sciences
National Chemical Laboratory, Pune - 411008

Ethanol is used for multiple purposes in chemical industries and is now considered as an alternate source of energy. In India it is produced by conventional batch fermentation using cane molasses. Improvement of yeast strains, fixation of optimum conditions in fermentation technology are under active investigation. Attempts have been made to recycle the yeast cell for ethanol production leading to a saving of 6 - 7% sugars required for inoculum. It has been found earlier in this laboratory that the rate of ethanol production was improved by skim milk supplement. Taking advantage of this observation, experiments were planned in yeast cell recycling fermentation using skim milk supplement. Three sets of fermentation were run (A) 15% total molasses reducing sugars + 0.25% urea + 0.05% $MgSO_4.7H_2O$. (B) Same as A + yeast extract (C) Same as A + 0.2% skim milk (100 ml in 150 ml conical flask). After completing first incubation for 48 h at 30°C, 90 ml fermented medium was removed by decantation, and fresh medium of similar composition was transferred for remaining six cycles by 24 h. After seven cycles it has been observed that 17.5% w/v more ethanol was formed in presence of skim milk supplement was compared to that without any supplement.

## IYG - P(I)6 CONTINUOUS FERMENTATION OF CASHEW APPLE JUICE

A.M.Raveendra, K.Venkataramu and S.P.Manjrekar
Discipline of Microbiology and Sanitation
Central Food Technological Research Institute, Mysore-13

With the availability of nearly one million tonnes of cashew-apple in this country, (the major part going waste) fermentation of the juice is a profitable proposition. Whereas batch fermentation and distillation results in the production of *feni* (mainly produced in Goa, continuous fermentation of cashew-apple juice with higher conversion rates, could result in a nutritious wine of acceptable quality.

Trials with a laboratory type continuous fermentor vessel, using *S.cerevisiae*, resulted in wine with 9% alcohol with a productivity of 1.56 to 1.83 g alcohol/l/hr, which is double that of the batch fermentation. Furthermore the fermentation time was reduced from 90 hr to between 30 and 60 hrs, depending on the dilution rates used for the continuous fermentation.

## IYG - P(I)7 PILOT SCALE BIOETHANOL PRODUCTION FROM SUGAR BEET IN INDIA

D.S.Dahiya, P.Tauro, A.K.Mittal and A. Bhargava
Dept. of Microbiology, H.A.U., Hisser and the
Ganga Nagar Sugar Mills, Ganga Nagar, Rajasthan.

In recent years, the demand for Bioethanol as a chemical feed stock as well as for potable purposes has been steadily increasing. However in India, bioethanol is produced mainly from sugar cane molasses which is not adequately available. It was, therefore considered necessary to explore alternate substrates for bioethanol production. Sugar beet which is a short duration crop, with a conversion efficiency near to sugar cane and with high yields, is a promising alternative to cane molasses particularly in states like Rajasthan, which does not have enough cane molasses, but has an ever increasing demand for bioethanol and imports alcohol from other states at high cost. We, therefore, extended the earlier work which was on lab scale, to pilot scale for the production of bioethanol from sugar beet. In this, the sugar beet was washed, sliced and extracted in a DDS diffuser for 4 to 6 hr. The slices were then pressed and the pressed juice in supplemented with urea, pH adjusted to 4-4.5 and charged to 16,000 lit fermenters. The juice was then inoculated at 10% level with an inoculum of *S. cerevisial - R* raised in beet juice and allowed to ferment at 35-37$^{0}$. From 1600 tons of beet 80,000 l of juice which had a brix of 12 was obtained. This after fermentation (30 hr) yielded about 4500 lit of bioethanol. The approximate cost of bioethanol production from sugar beet is about Rs. 4-5/bulk l. which is much higher than the production from sugar cane molasses. Yet, considering the cost at which it is imported from other states, the production of ethanol from beet appears attractive only in states which are at present importing

ethanol from other states. The production cost can however be reduced by increasing the level of sugar in the mash and by efficient utilization of the waste pulp as cattle feed.

## IYG - P(I)8 ETHYL ALCOHOL PRODUCTION FROM TWO UNUSUAL SUBSTRATES

Lokendra Singh
Defence Food Research Laboratory, Mysore - 11

The present shortage and likely exhaustion of the petroleum products have necessitated a continued search for some alternative sources of energy. Being an agricultural country we are rich in cellulosic wastes. Therefore ethanol production from these cellulosic waste materials appears to be a viable approach to this problem. During the present studies corn cob and groundnut shells thrown away as discards were taken up for the investigation. Their cellulose content varies from 33-38.8%. Various treatments were studied and treatment of the powdered substrates with HCl and $H_2SO_4$ caused 88-97% saccharification in corn cob and 69-75% saccharification in ground nut shell. The cellulase enzyme from *T. viride* QM 9414 resulted in poor yield of reducing sugars. Fermentation studies were conducted with *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* NCYC 240 and *S.cerevisiae* var *ellipsoideus* out of which the last strain yielded highest amount of alcohol at the end of 24 hrs fermentation at 30°C.

## IYG - P(I)9 COMPARISON OF SOLID AND LIQUID STATE FERMENTATION OF SUGARCANE AND SUGARBEET TO ETHANOL

S.Nirmala Devi and S.C.Basappa
Central Food Technological Research Institute
Mysore-570 013

Fermentation of whole pulp and juice of sugarcane and sugarbeet separately to ethanol by *Saccharomyces cerevisiae* 101 was carried out. This was done with the main objective of obtaining maximum concentration as well as yield of ethanol from the sugars present in these raw materials.

The study revealed that not only the overall yield of ethanol from pulp (solid) fermentation was less but also the concentration of ethanol obtained for distillation was lower (8% W/V), mainly due to slow and incomplete fermentation of sugars, than that obtained in liquid fermentation (10.24 W/V). The biomass production was also more ($9.8 \times 10^{10}$ cells) in the case of solid state fermentation as compared to liquid fermentation ($6.3 \times 10^{10}$ cells) Continuous cell-immobilized fermentation of juice in an U-shaped reactor could be easily performed.

Based on the data obtained it was concluded that the juice fermentation was better (99%) than pulp (solid) fermentation (79%) in terms of theoretical yield and the rate of fermentation as well as overall yield of ethanol from available sugars in sugarcane and sugarbeet.

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## IYG - P(II)1 DEVELOPMENT OF AN UV-MUTANT OF ZYMOMONAS MOBILIS ZM 4 FOR IMPROVED ETHANOL PRODUCTION

O.Sreekumar and S.C. Basappa
Central Food Technological Research Institute, Mysore-570 013

Exposure of *Z. mobilis* ZM 4 cells to shortwave UV light induced mutants that were better ethanol producers than the wild type. A selected mutant was characterized for thermotolerance, ethanol-tolerance and osmo-tolerance as well as for rate and yield of ethanol production in comparison with the wild type at 37°C and pH 5.0 in glucose-yeast extract medium.

It was found that the mutant (ZM 401) not only gave better yield (g/g of sugar) of ethanol from glucose, faster rate of ethanol production (g of ethanol/h) but also less biomas (g/g of glucose) than those of the wild type. This was evident from the results that the mutant produced less of lactic and acetic acids than those of the wild type.

The mutant performed better than wild type at 37°C, it tolerated more sugar (38%) and ethanol (7%) under experimental conditions. Its activity at 37°C was similar to the one at 30°C.

## IYG - P(II)2 ETHANOL PRODUCTION FROM LACTOSE USING A CO-CULTURE OF ZYMOMONAS MOBILIS 4286 and KLUYVEROMYCES FRAGILIS 665

N.R.Kamini, T.Karunakaran, H.Nelliah and P.Gunasekaran
Department of Microbiology, School of Biological Sciences, Madurai Kamaraj University, Madurai - 625 021.

*Zymomonas mobilis*, a gram negative bacterium, has several advantages over the traditional ethanol producing yeast but the utilizable substrate range is limited to glucose, fructose and sucrose. *Z.mobilis* NRRL B-4286 was found to be an efficient strain when compared to the strains already reported. This strain is co-culture with *Kluyveromyces fragilis* NRRL 665, using an adapted batch fermentation, produced 64.4 g/l and 66 g/l ethanol from 200 g/l and 250 g/l lactose respectively.

These values are higher than those reported already from our laboratory using a co-culture of *Z.mobilis* NRRL B-1960 and *K.fragilis* 665. Contrary to the results of Vienne and Von Stocker, adapted *K.fragilis* 665 produced 59.2 g/l ethanol from 250 g/l lactose in mono-culture. In other words, adaptation enhanced the ethanol tolerance of *K.fragilis*. However, the parameters related to growth and sugar uptake were affected on increasing the initial lactose concentration. In addition to these, ethanol production using co-immobilization of *Z.mobilis* 4286 and *K.fragilis* 665 had also been tried. Developing this fermentation process would be of help to derive ethanol from whey permeate in future.

## IYG - P(II)3 PHYSIOLOGICAL STUDIES OF OVER-EXPRESSION OF ALDOLASE IN YEAST

Z.Lobo, R.Mehta and P.K.Maitra
Tata Institute of Fundamental Research, Bombay 400005

The structural gene of fructose 1, 6-bisphosphate aldolase of *Saccharomyces cerevisiae* has been cloned. A cDNA library of wild-type strain in a yeast expression vector was used to transform a glucose-negative aldolase mutant. The insert size of 1.2 kb corresponds to the known size of the aldolase monomer. This high-copy plasmid increases the aldolase activity in the crude extracts of yeast to about 5 times the level in the wild-type strain. However, the glycolytic rate remains unaffected. The increased enzyme activity in the transformant causes a pronounced rise in the levels of both the triose phosphates. The results are consistent a rate-limitation at the subsequent step catalysed by glyceraldehyde 3-P dehydrogenase. The highly active triose phosphate isomerase of the yeast cell fails to keep pace with the increased aldolase activity in cells bearing the cloned gene.

## IYG - P(II)4 ISOLATION OF EXTRACHROMOSOMAL DNA FROM HYDROCARBON UTILISING YEAST STRAIN

G.N.Qazi, R.Prashad, V.Verma and C.L.Chopra
Regional Research Laboratory, Jammu-Tawi - 180001

Ethyl alcohol still remains as one of the major biotechnological products and yeast happens to be the main organism producing this solvent using waste carbohydrate material. A relatively recent development for exploitation of yeasts for industrial use has been SCP, organic and amino acids and vitamin production using refinery wastes/by-products like paraffins. A strain of *Candida lipolytica* isolated and developed for the production of citric acid from paraffin was investigated for the presence of extrachromosomal DNA. The protocol used for its isolation and characterisation are described. Electron microscopic examination and restriction digestion of the plasmid DNA from the hydrocarbon utilising strain of *Candida lipolytica* were also carried out.

## IYG - P(II)5 HYDROCARBON FEED STOCK FOR YEAST PROPAGATION AND CITRIC ACID PRODUCTION

G.N.Qazi and C.L. Chopra
Regional Research Laboratory, Jammu-Tawi-180001

*Yerrowia lipolytica* as the organism and n-alkanes as the potential carbon source has been investigated for citric acid production. Various n-alkanes were used to study the kinetics of citric acid production. The effect of substrate uptake on cell growth and yeild co-efficients was documented under varied oxygen transfer rates in shape flasks and laboratory fermenters.

## IYG - P(II)6 STUDIES ON YEAST ISOLATES FROM AYURVEDIC PREPARATIONS AND DHAYATI FLOWERS

N.R.Shirsat, Z.H.Bengali, and S.P.Manjrekar
Food Technology Section
University Department of Chemical Technology, Bombay-19

*Asavas* and *Arishtas* are natural Ayurvedic fermented products with high medicinal value. Several plant materials and their extracts serve as the source of micro-organisms and active constituents in these fermentations.

Attempts were made to isolate and identify the yeast species from some unpasteurized Ayurvedic preparations and *Dhayati (Woodfordia floribunda)* flowers, which are used for initiating these alcoholic fermentations.

Out of the ten isolates obtained, six were identified as *Saccharomyces steineri*, two as *Torulopsis* spp. and one each, as *S.cerevisiae and Rhodotorula mucilogenosa.*

*S.steineri* was able to tolerate and grow in sugar and alcohol concentrations of 50% and 15% respectively.

## IYG - P(II)7 FLUCTUATION OF YEAST POPULATION IN NEERA COLLECTED AT VARYING pH LEVELS

A.D.Ravindran, N.Sivakumar, S.Ravanappa and P.V.Subramanian
Faculty of Rural Oriented Sciences, Gandhigram Rural Institute
Gandhigram-624302, Tamil Nadu.

Neera is a sweet nutritive juice obtained by tapping palm trees *(Borassus flabellifermis)*. The present study was undertaken with a view to observe the fluctuation of yeast populations in the neera collected at five different pH levels (pH 7.5 to 9.5). It was observed that the population of yeast varied from $x10^2$ to $10^6$ per ml of neera according to the pH level of the collected juice. A gradual increase in the population of yeast was observed from the start of the season in December reaching its peak in June. Reasons which could be attributed to this randomize microbial make up are variability of palm sap. seasonal variations (difference in ambient temperature) and tapping procedures.

When the pH of neera was below 8.0, within 48 hr more than half of the total sugars were fermented (Ethanol concentrations- 5.0 to 5.2% v/v) reducing the initial pH of neera to 4.0 to 4.3. Another problem encountered in preservation of neera is the production of hydrogen sulphide (150 moles/lit) by certain wild yeast strains.

## IYG - P(II)8 OPTIMIZATION OF YIELD AND ACTIVITY OF BAKER's YEAST FROM MOLASSES IN 10 L FERMENTOR

G.Vijayalakshmi and S.C.Basappa
Central Food Technological Research Institute, Mysore - 570 013

The optimum conditions such as aeration rate (1.5 VVM), agitation speed (300 rpm), pH (4.5) and temperature (28-30°C) increased the yield, trehalose content and fermenting power of both wet yeast (WY) and active dry yeast (ADY). A fed-batch process yielded more yeast with better activity than that of incremental fe-ding of molasses in 10 L fermentor. Alteration of pH and temperature at the end of fermentation contributed to more trehalose and better activity of yeast. Urea as nitrogen source had many advantages. Addition of certain concentrations of biotin and stearic acid during fermentation enhanced the activity and trehalose content of ADY.

Supplementation of certain carbohydrates and other compounds during ripening stage of fermentation significantly increased trehalose content of yeast but not glycogen. Glucan content of yeast increased after drying only in some cases. However, mannan did not increase in most of the cases. Addition of trehalose and lecithin imparted maximum activity in ADY.

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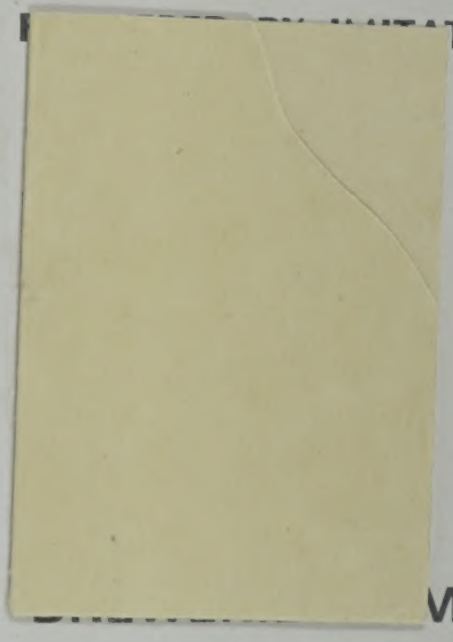

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